

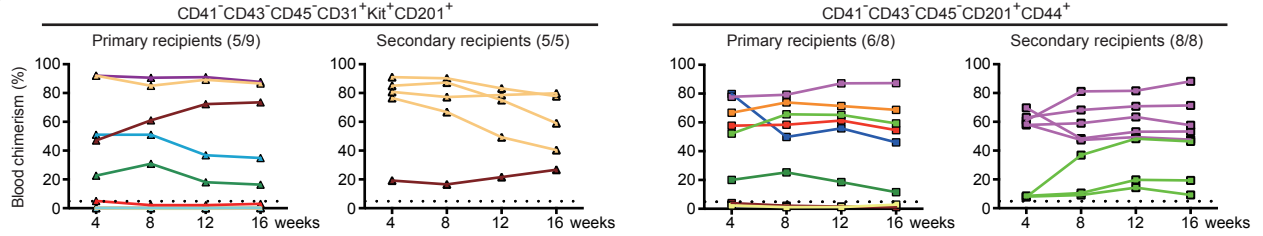
**Fig. S2**

**a**

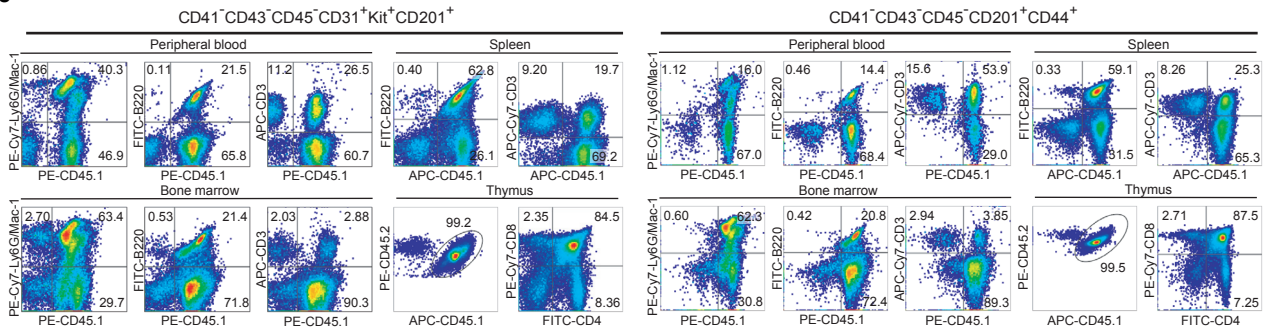
| Stage                               | Region      | Exp (n) | Population  | Constitution in the region (%) | Cell dose <sup>a</sup>  | Clusters <sup>b</sup> |
|-------------------------------------|-------------|---------|---|--------------------------------|-------------------------|-----------------------|
| E9.5-E10.0 (30-32 sp <sup>c</sup> ) | Caudal half | 4       | CD41 <sup>-</sup> CD43 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> Kit <sup>+</sup> CD201 <sup>+</sup> | 0.20±0.07                      | 3.0-4.0 ee <sup>d</sup> | 13/13 (100%)          |
|                                     |             |         | CD41 <sup>-</sup> CD43 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> Kit <sup>-</sup> CD201 <sup>-</sup> | 1.25±0.45                      | 3.0-4.0 ee              | 2/13 (15.4%)          |
| E9.5-E10.0 (30-34 sp)               | Caudal half | 4       | CD41 <sup>-</sup> CD43 <sup>-</sup> CD45 <sup>-</sup> CD201 <sup>+</sup> CD44 <sup>+</sup>                  | 0.09±0.03                      | 3.0-4.0 ee              | 11/11 (100%)          |
|                                     |             |         | CD41 <sup>-</sup> CD43 <sup>-</sup> CD45 <sup>-</sup> CD201 <sup>+</sup> CD44 <sup>-</sup>                  | 0.33±0.21                      | 3.0-4.0 ee              | 0/11                  |

<sup>a</sup>cell dose per well and per recipient. <sup>b</sup>hematopoietic cluster positive wells/total wells. <sup>c</sup>somite pairs. <sup>d</sup>embryo equivalents.

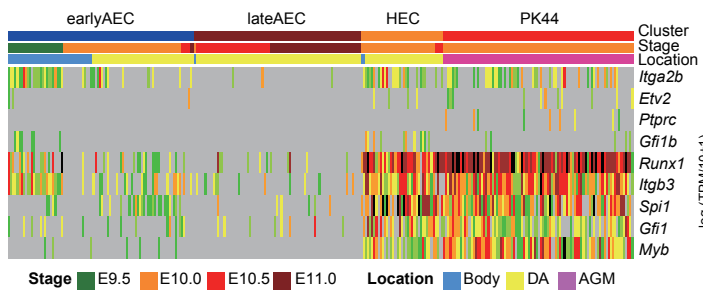
**b**



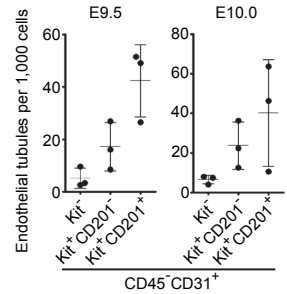
**c**



**d**



**e**



**f**

| Stage                               | Region      | Exp (n)        | Population in EC (CD41 <sup>-</sup> CD43 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> ) | Constitution in the region (%) | Cell dose <sup>a</sup> | Endothelial only <sup>b</sup> | Endothelial and hematopoietic <sup>c</sup> | Hematopoietic only <sup>d</sup> |
|-------------------------------------|-------------|----------------|---|--------------------------------|------------------------|-------------------------------|--|---------------------------------|
| E9.5-E10.0 (29-31 sp <sup>e</sup> ) | Caudal half | 2 <sup>f</sup> | Kit <sup>+</sup> CD201 <sup>+</sup> CD44 <sup>+</sup>                                       | 0.11±0.03                      | 10 cells               | 0/44                          | 35/44                                      | 9/44                            |
|                                     |             |                | Kit <sup>+</sup> CD201 <sup>+</sup> CD44 <sup>-</sup>                                       | 0.10±0.02                      | 10 cells               | 33/42                         | 0/42                                       | 0/42                            |
| E9.5-E10.0 (30-32 sp)               | AGM         | 5 <sup>g</sup> | Kit <sup>+</sup> CD201 <sup>+</sup> CD44 <sup>+</sup>                                       | 0.31±0.09                      | Single cell            | 60/261 (1:4)                  | 7/261 (1:37)                               | 106/261 (1:2)                   |

<sup>a</sup>number of cells per well. <sup>b</sup>only endothelial tube positive wells/total wells. <sup>c</sup>both endothelial and hematopoietic progeny positive wells/total wells. <sup>d</sup>only hematopoietic progeny positive wells/total wells. <sup>e</sup>somite pairs. <sup>f</sup>2 independent experiments, totally 12 embryos were used. <sup>g</sup>5 independent experiments, totally 15 embryos were used.

**Supplementary Figure 2.** Identification of the HSC-competent and endothelial-hematopoietic dual-potent HECs. **a.** Detailed information of the co-culture/transplantation assays performed with E9.5-E10.0 caudal half cells. **b.** Blood chimerism of the primary and secondary recipients at 4-16 weeks post-transplantation. The primary recipients were transplanted with the derivatives of the indicated cell populations from the caudal half of E9.5-E10.0 embryos. The paired primary and corresponding secondary repopulated mice are shown as the same symbol and color. Numbers of repopulated/total recipients are shown in the brackets. Only the recipients survived to 16 weeks post-transplantation are shown. **c.** FACS plots showing representative primary recipients with long-term (16 weeks), multi-organ and multi-lineage repopulations transplanted with the derivatives of the indicated cell populations from the caudal half of E9.5-E10.0 embryos. Donor-derived (CD45.1<sup>+</sup>CD45.2<sup>+</sup>) myeloid (Gr-1<sup>+</sup>/Mac-1<sup>+</sup>), B lymphoid (B220<sup>+</sup>), and T lymphoid (CD3<sup>+</sup>) cells in multiple hematopoietic organs are shown. **d.** Heatmap showing the expression of selected genes in earlyAEC, lateAEC, HEC and PK44 populations. Note the similarity of expression patterns between HEC and PK44. **e.** Graph showing the endothelial potential of different cell populations in E9.5-E10.0 body part of embryo proper. Cells with indicated immunophenotype were isolated by FACS, co-cultured with OP9 stromal cells for 7 days, and stained with CD31 to identify the endothelial tubes. Data are means  $\pm$  s.d.. For E9.5 embryos, data are from 3 independent experiments with 6-9 embryo equivalents pooled for each experiment. For E10.0 embryos, data are from 3 independent experiments with 8-9 embryo equivalents pooled for each experiment. **f.** Detailed information of endothelial-hematopoietic dual-potential induction assays performed with cells from E9.5-E10.0 caudal half or AGM region.